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(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

118.13-US-WO

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/856114

INTERNATIONAL APPLICATION NO.

PCT/KR99/00699

INTERNATIONAL FILING DATE

19 November 1999 (19.11.99)

PRIORITY DATE CLAIMED

19 November 1998 (19.11.98)

TITLE OF INVENTION HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV AND  
PREPARATION METHOD THEREOF

APPLICANT(S) FOR DO/EO/US


Hyo Jeong Hong, Chun Jeih Ryu, and Hyangsuk Hur

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☐ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

## Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
  - (a) Computer Readable and Paper Form of Sequence Listing

|  |              |   |                   |
|--|--------------|---|-------------------|
| U.S. APPLICATION NO. <b>09/856114</b><br>INTERNATIONAL APPLICATION NO.<br>PCT/KR99/00699   |              | ATTORNEY'S DOCKET NUMBER<br>118.13-US-WO  |                   |
| 21. <input checked="" type="checkbox"/> The following fees are submitted:<br><b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b><br>Neither international preliminary examination fee (37 CFR 1.482)<br>nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO<br>and International Search Report not prepared by the EPO or JPO. .... <b>\$1000.00</b><br><br>International preliminary examination fee (37 CFR 1.482) not paid to<br>USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b><br><br>International preliminary examination fee (37 CFR 1.482) not paid to USPTO<br>but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b><br><br>International preliminary examination fee (37 CFR 1.482) paid to USPTO<br>but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b><br><br>International preliminary examination fee (37 CFR 1.482) paid to USPTO<br>and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b><br><br><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b> |              | <b>CALCULATIONS PTO USE ONLY</b>  |                   |
| Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30<br>months from the earliest claimed priority date (37 CFR 1.492(e)).  |              | \$ 1000.00  |                   |
| CLAIMS   | NUMBER FILED | NUMBER EXTRA  | RATE              |
| Total claims   | 15 - 20 =    |   | x <b>\$18.00</b>  |
| Independent claims   | 6 - 3 =      | 3   | x <b>\$80.00</b>  |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable)  |              |   | + <b>\$270.00</b> |
| <b>TOTAL OF ABOVE CALCULATIONS =</b>   |              | \$ 1240.00  |                   |
| <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above<br>are reduced by 1/2.  |              | +   |                   |
| <b>SUBTOTAL =</b>  |              | \$ 1240.00  |                   |
| Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30<br>months from the earliest claimed priority date (37 CFR 1.492(f)).   |              |   |                   |
| <b>TOTAL NATIONAL FEE =</b>  |              | \$ 1240.00  |                   |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be<br>accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +   |              |   |                   |
| <b>TOTAL FEES ENCLOSED =</b>   |              | \$ 1280.00  |                   |
|  |              | Amount to be<br>refunded:   | \$                |
|  |              | charged:  | \$                |
| a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed.<br>b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.<br>A duplicate copy of this sheet is enclosed.<br>c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any<br>overpayment to Deposit Account No. <u>50-0494</u> . A duplicate copy of this sheet is enclosed.<br>d. <input checked="" type="checkbox"/> Fees are to be charged to a credit card. <b>WARNING:</b> Information on this form may become public. <b>Credit card</b><br><b>information should not be included on this form.</b> Provide credit card information and authorization on PTO-2038.   |              |   |                   |
| <b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.</b>  |              |   |                   |
| SEND ALL CORRESPONDENCE TO:<br>Karen S. Canady<br>Gates & Cooper LLP<br>6701 Center Drive West, Suite 1050<br>Los Angeles, CA 90045  |              |   |                   |
|  |              | <br>SIGNATURE |                   |
|  |              | Karen S. Canady<br>NAME   |                   |
|  |              | 39,927<br>REGISTRATION NUMBER   |                   |

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Hyo Jeong Hong et al. Examiner: To be assigned  
Serial No.: To be assigned Group Art Unit: To be assigned  
Filed: To be assigned Docket: 118.13-US-WO  
Title: HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF  
HBV AND PREPARATION METHOD THEREOF

## CERTIFICATE OF MAILING UNDER 37 CFR 1.10

'Express Mail' mailing label number: EL816010064US

Date of Deposit: May 17, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

By: Darlene Ross

Name: Darlene Ross

PRELIMINARY AMENDMENT

BOX PCT

Commissioner for Patents

Washington, D.C. 20231

Dear Sir:

Prior to a first Office Action, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please amend the specification as follows:

At page 1, after the Title, please insert the following paragraph:

--This application claims the benefit of Korean Patent Application No. 1998-49663, filed

November 19, 1998.—

IN THE CLAIMS

Please cancel claims 1-15 without prejudice, and add new claims 16-30 as follows:

16. (NEW) A humanized antibody specific for HBV surface antigen pre-S1, containing a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 20.

17. (NEW) A humanized antibody specific for HBV surface antigen pre-S1, containing a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 21.

18. (NEW) The humanized antibody of claim 17, wherein the amino acid sequence of the humanized heavy chain variable region is modified from SEQ ID NO: 21 by at least one amino acid substitution selected from the group comprising:

Lys<sup>12</sup> → Val<sup>12</sup>,      Thr<sup>28</sup> → Ala<sup>28</sup>,      Thr<sup>30</sup> → Ser<sup>30</sup>,  
Met<sup>48</sup> → Ile<sup>48</sup>,      Arg<sup>67</sup> → Lys<sup>67</sup>,      Val<sup>68</sup> → Ala<sup>68</sup>,  
Met<sup>70</sup> → Leu<sup>70</sup>,      Val<sup>79</sup> → Ala<sup>79</sup>,      and Tyr<sup>95</sup> → Phe<sup>95</sup>.

19. (NEW) A humanized antibody specific for HBV surface antigen pre-S1, containing a humanized light chain variable region which comprises an amino acid sequence described by SEQ ID NO: 23.

20. (NEW) A gene encoding a humanized heavy chain which comprises a humanized heavy chain variable region having an amino acid sequence selected from the group consisting of: the amino acid sequence described by SEQ ID NO: 20; the amino acid sequence described by SEQ ID NO: 21; and the amino acid sequence of SEQ ID NO: 21 which is modified by at least one amino acid substitution

selected from the group comprising: Lys<sup>12</sup> → Val<sup>12</sup>, Thr<sup>28</sup> → Ala<sup>28</sup>, Thr<sup>30</sup> → Ser<sup>30</sup>, Met<sup>48</sup> → Ile<sup>48</sup>, Arg<sup>67</sup> → Lys<sup>67</sup>, Val<sup>68</sup> → Ala<sup>68</sup>, Met<sup>70</sup> → Leu<sup>70</sup>, Val<sup>79</sup> → Ala<sup>79</sup>, and Tyr<sup>95</sup> → Phe<sup>95</sup>.

21. (NEW) The gene of claim 20, wherein the humanized heavy chain variable region comprises an amino acid sequence described by SEQ ID NO: 20.

22. (NEW) The gene of claim 20, wherein the humanized heavy chain variable region comprises an amino acid sequence described by SEQ ID NO: 21.

23. (NEW) A gene encoding a humanized light chain which contains a humanized light chain variable region comprising an amino acid sequence described by SEQ ID NO: 23.

24. (NEW) An expression vector containing the gene of claim 20.

25. (NEW) The expression vector of claim 24, which is designated pCMV-HKR127HC (Accession Number: KCTC 0531BP).

26. (NEW) The expression vector of claim 24, which is designated pCMV-HKR127(III)HC (Accession Number: KCTC 0691BP).

27. (NEW) An expression vector containing the gene of claim 23.

28. (NEW) The expression vector of claim 27, which is designated pKC-dhfr-HKR127 (Accession Number: KCTC 0529BP).

29. (NEW) A pharmaceutical composition containing a humanized antibody specific for HBV surface antigen pre-S1, wherein the humanized antibody comprises:

- (a) a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 20,
- (b) a humanized heavy chain variable region which comprises an amino acid sequence described by SEQ ID NO: 21;
- (c) a humanized heavy chain variable region which comprises an amino acid sequence of SEQ ID NO: 21 which is modified by at least one amino acid substitution selected from the group comprising: Lys<sup>12</sup> → Val<sup>12</sup>, Thr<sup>28</sup> → Ala<sup>28</sup>, Thr<sup>30</sup> → Ser<sup>30</sup>, Met<sup>48</sup> → Ile<sup>48</sup>, Arg<sup>67</sup> → Lys<sup>67</sup>, Val<sup>68</sup> → Ala<sup>68</sup>, Met<sup>70</sup> → Leu<sup>70</sup>, Val<sup>79</sup> → Ala<sup>79</sup>, and Tyr<sup>95</sup> → Phe<sup>95</sup>, or
- (d) a humanized light chain variable region comprising an amino acid sequence described by SEQ ID NO: 23

30. (NEW) A method of protecting against HBV infection or treating chronic hepatitis B, comprising administering the pharmaceutical composition of claim 29.

#### REMARKS

Prior to a first Office Action in this application, Applicants request that original claims 1-15 be canceled without prejudice, and that new claims 16-30 be added. These new claims do not involve

any new matter or objectionable changes. When the Examiner takes this application up for action, it is requested that the foregoing be taken into account.

It is submitted that this application is now in good order for allowance and such allowance is respectfully solicited. Should the Examiner believe minor matters still remain that can be resolved in a telephone interview, the Examiner is urged to call Applicants' undersigned attorney.

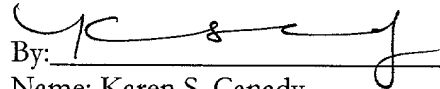
Respectfully submitted,

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By their attorneys,

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Date: May 18, 2001

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G&C 118.13-US-WO

Rec'd PCT/PTO 18 MAY 2001

HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1  
OF HBV AND PREPARATION METHOD THEREOF

FIELD OF THE INVENTION

5           The present invention relates to humanized antibodies specific for HBV surface antigen pre-S1.

Particularly, this invention relates to humanized antibodies specific for HBV surface antigen pre-S1, the antibody comprising humanized heavy and light chain; to  
10 genes encoding the humanized heavy or light chain; to expression vectors containing said genes and *E. coli* transformants containing said expression vector; and to pharmaceutical composition comprising said humanized  
15 antibody, which may be administered in order to prevent HBV infection or to treat chronic hepatitis B.

BACKGROUND

HBV (Hepatitis B Virus) is responsible for chronic or acute human hepatitis that may get worse to liver  
20 cirrhosis or cancer. It is estimated that about three hundred million people are suffering from hepatitis in the world (Tiollais and Buendia, *Sci. Am.* 264:48, 1991).

There are three kinds of HBV surface proteins containing different sets of surface antigens.  
25 Particularly, these surface antigen proteins includes the Major Protein containing S antigen, the Middle



Protein containing S and pre-S2 antigens, and the Large Protein containing S, pre-S2 and pre-S1 antigens (Neurath and Kent, *Adv. Virus Res.*, 34:65-142, 1988). All the surface antigen proteins can induce antibodies that neutralize HBV, and especially, antibodies against HBV pre-S antigen are associated with the elimination of the virus and the recovery from HBV infection, overcoming non-responsiveness to the S antigen (Iwarson et al., *J. Med. Virol.*, 16:89-96, 1985; Itoh et al., *Proc. Natl. Acad. Sci. USA*, 85:9174-9178, 1986; Budkowska et al., *J. Med. Virol.*, 20:111-125, 1986; Milich et al., *Proc. Natl. Acad. Sci. USA*, 82:8168-8172, 1985; Milich et al., *J. Immunol.*, 137:315-322, 1986).

Unlike pre-S2 or S antigen, pre-S1 antigen is exclusively present in infectious virus particles (Heerman et al., *J. Virol.*, 52:396-402, 1984) and involved in the infection into human liver cells. Thus, it has been reported that monoclonal antibody specific for pre-S1 antigen may efficiently neutralize HBV (Neurath et al., *Cell*, 46:429, 1986; Pontisso et al., *Virology*, 173:533, 1989; Neurath et al., *Vaccine*, 7:234, 1989), and the monoclonal antibody is considered to be useful in the prevention of HBV infection and the treatment of chronic hepatitis B.

So far hepatitis B immunoglobulin has been employed as a preventive for HBV infection, which may

protect, for example, a newborn baby from a HBV-positive mother, medical personnel exposed to HBV, and liver transplant patient with chronic HBV-related liver disease (Beasley et al., *Lancet*, 2:1099, 1983; Todo et al., *Hepatology*, 13:619, 1991). However, hepatitis B immunoglobulin has some shortcomings such as its limited availability, low specific activity and its possible contamination with infectious agents. Furthermore, it is another disadvantage of hepatitis B immunoglobulin that blood plasma should be continuously supplied.

As an alternative for the hepatitis B immunoglobulin, mouse monoclonal antibodies against HBV surface antigens have been developed. Although the mouse monoclonal antibodies show high affinity for the antigen and can be prepared on a large scale, they induce human anti-mouse antibody response in patients (Shawler et al., *J. Immunol.*, 135:1530, 1985). There were attempts to prepare human monoclonal antibodies, but few of these antibodies showed a high level of affinity.

Instead, humanized antibodies have been developed. Humanized antibody has a high level of affinity and specificity similar to mouse antibodies, whereas its immunogenicity is minimized. Humanized antibody is a hybrid antibody in which CDRs (Complementarity Determining Regions) of a mouse antibody is grafted to

a human antibody by genetic engineering technique. It can be easily prepared on a large scale, and hardly elicits immune responses in humans since most of the DNA sequences encoding the humanized antibodies are  
5 derived from a human DNA sequence (Riechman et al., *Nature*, 332:323, 1988; Nakatani et al., *Protein Engineering*, 7:435, 1994).

To overcome the aforementioned and other  
10 disadvantages of mouse or human HBV immunoglobulin, we, the inventors of the present invention, have attempted to prepare humanized antibodies which can be used to prevent HBV infection and to treat chronic hepatitis B. Prior to this invention, we prepared a mouse monoclonal  
15 antibody KR127 against HBV surface antigen pre-S1. Additionally, we isolated the genes encoding the heavy and light chain variable regions of KR127 antibody and determined the sequences of the genes (Korea Patent Application No. 1997-30696). The present invention is  
20 performed by selecting human immunoglobulin genes homologous to the sequences of KR127 antibody light chain and heavy chain variable regions; constructing the humanized antibody genes; inserting the genes into expression vectors; introducing the vectors into host  
25 cells; obtaining humanized antibodies from the culture of the transformed cells; and confirming that the humanized antibodies have high affinity to HBV pre-S1

antigen, similar to the mouse monoclonal antibody KR127.

#### SUMMARY OF THE INVENTION

It is an object of this invention to provide  
5 humanized antibodies specific for CDRs of mouse HBV  
surface antigen pre-S1, having high affinity to the  
antigen and reduced immunogenicity in human.

In accordance with the present invention, the  
10 foregoing objects and advantages are readily obtained.

The present invention provides humanized  
antibodies specific for HBV surface antigen pre-S1,  
comprising humanized heavy and light chains.

This invention also provides genes encoding the  
15 variable regions of said humanized heavy or light chain.

In addition, this invention provides expression  
vectors containing said genes and *E. coli* transformants  
containing said expression vectors.

This invention further provides pharmaceutical  
20 compositions comprising said humanized antibody, which  
may be administered in order to prevent HBV infection  
or to treat chronic hepatitis B.

Further features of the present invention will  
appear hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1a and FIG 1b comparatively depict the amino acid and nucleotide sequences of  $V_H$  regions (for Variable regions of Heavy chains) in a mouse monoclonal antibody KR127 and in two humanized antibodies of this invention,

FIG 2a schematically depicts a process for preparing HKR127HC(I) gene encoding the heavy chain of a humanized antibody of this invention,

FIG 2b schematically depicts a process for preparing HKR127HC(III) gene encoding the heavy chain of a humanized antibody of this invention,

FIG 3a and FIG 3b comparatively depict the amino acid and nucleotide sequences of  $V_L$  regions (for Variable regions in Light chain) in a mouse monoclonal antibody KR127 and in a humanized antibody of this invention.

FIG 4 schematically depicts a process for preparing HKR127KC(I) gene encoding a humanized antibody of this invention,

FIG 5a depicts an expression vector pCMV-HKR127HC containing a gene for heavy chain of the humanized antibody,

FIG 5b depicts an expression vector pKC-dhfr-HKR127 containing a gene for light chain of the humanized antibody,

FIG 5c depicts an expression vector pCMV-HKR127HC(III) containing a gene for heavy chain of the humanized antibody,

FIG 6a comparatively shows the binding affinities of a humanized antibody (HZKR127I) and a mouse monoclonal antibody (KR127), and

FIG 6b comparatively shows the binding affinities of a humanized antibody (HZKR127III) and a humanized antibody (HZKR127I).

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Hereinafter, the present invention is described in detail.

This invention provides humanized antibodies specific for HBV surface antigen pre-S1, comprising humanized heavy and humanized light chains.

This invention also provides genes encoding the variable regions of said humanized heavy or light chain.

Said humanized heavy chain contains variable region which is derived from the  $V_H$  region of mouse KR127 antibody. The  $V_H$  region of mouse KR127 antibody is described by SEQ ID NO: 19, and the  $V_H$  region of the humanized antibody of this invention can be prepared by grafting the CDRs of mouse KR127  $V_H$  region to homologous human immunoglobulin  $V_H$  region.

And said humanized light chain contains variable

region which is derived from the  $V_L$  region of mouse KR127 antibody. The  $V_L$  region of mouse KR127 antibody is described by SEQ ID NO: 22, and the  $V_L$  region of the humanized antibody of this invention can be prepared by grafting the CDRs of mouse KR127  $V_L$  region to homologous human immunoglobulin  $V_L$  region.

In preferred embodiments, we screened human immunoglobulin that show the highest similarities of amino acid sequence to the heavy or light chain of the mouse monoclonal antibody KR127. In result, human immunoglobulin germ line genes DP7 and DPK12 were screened from GenBank database. DP7 shows the highest homology to the  $V_H$  region of mouse antibody KR127, while DPK12 is most similar to the  $V_L$  region of KR127.

The humanized antibodies of this invention can be produced from recombinant genes encoding humanized  $V_H$  region or  $V_L$  region. These genes are constructed by substituting CDRs of mouse KR127 for those of the human DP7 or DPK12 antibody. In constructing these genes, most of the amino acid residues corresponding to the humanized CDRs are derived from the CDRs of mouse antibody KR127. However, some mouse-derived CDRs residues are replaced by human counterparts, since their corresponding amino acid residues are expected not to be involved in the antigen binding (see FIG 1). In the same way, some human-derived amino acid residues for the non-CDR framework regions (FR) of variable

region are replaced with mouse counterparts, since it is expected that these FR residues may affect the conformation of CDRs.

Particularly, HKR127HCv(HZII) gene encoding a humanized V<sub>H</sub> region was prepared by grafting the partial CDR1, 2, 3 and a FR residue (at position 72) of mouse KR127 heavy chain to the human DP7 gene (see FIG 1).

However, antibody expressed from HKR127HCv(HZII) gene did not show any significant level of binding capacity to corresponding antigen. To improve the HKR127HCv(HZII) gene, we also prepared HKR127HCv(HZI) gene and HKR127HCv(HZIII) gene which contain more mouse-derived codons than HKR127HCv(HZII) gene (see FIG 1).

HKR127HCv(HZI) contains CDR1, partial CDR2, and CDR3, and 11 FR residues of mouse KR127 V<sub>H</sub>, while HKR127HCv(HZIII) contains the same mouse CDR codons and 2 mouse FR residues (see FIG 1).

To construct HKR127HC(I) gene encoding a full-length heavy chain of the humanized antibody of this invention, PCRs were conducted, in which template for amplification is either the HKR127HCv(HZII) gene or pRC/CMV-HC-HuS (KCTC 0229BP) containing the heavy chain leader sequence and the constant region sequence of human immunoglobulin heavy chain  $\gamma 1$ .

Six pairs of oligonucleotides (SEQ ID NO: 1 and 2;



3 and 4; 5 and 6; 7 and 8; 9 and 10; and 11 and 12) were used as PCR primers (see FIG 2a).

The first five PCR products were brought to annealing reaction. Then, the DNA fragment containing the five PCR products was employed as a template of recombinant PCR wherein two primers described by SEQ ID NO: 1 and 10 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12. The final 1431-bp PCR product, HKR127HC(I), encoding the heavy chain of a humanized antibody (HZKR127I) was introduced into pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(I).

The primers are described in SEQ ID NO: 1 to 12 in SEQUENCE LISTING, and particularly, primer described by SEQ ID NO: 1 contains EcoRI sequence at the 5' end, while primer described by SEQ ID NO: 12 does SalI sequence at the 3' end.

The variable region in the HKR127HC(I) gene contains 11 mouse-derived FR residues at positions 12, 28, 30, 48, 67, 68, 70, 72, 74, 79 and 95 (see FIG 1). The heavy chain variable region has 87 FR residues, and the unmodified FR residues is 76. Thus, the amino acid sequence of the heavy chain variable FR of the

HKR127HC(I) gene is 87% homologous to that of human DP7 gene.

To more humanize the HZKR127(I), HZKR127(III) gene was constructed, which contains HKR127HCv(HZIII) gene with 2 mouse-derived FR residues at position 72 and 74 (see FIG 1).

To construct HKR127HC(III) gene encoding a full-length heavy chain of the humanized antibody of this invention, PCRs were conducted, in which template for amplification is either the HKR127HCv(HZII) gene or pRC/CMV-HC-HuS (KCTC 0229BP) containing the heavy chain leader sequence and the constant region sequence of human immunoglobulin heavy chain  $\gamma 1$  (see FIG 2b).

Four pairs of oligonucleotides (SEQ ID NO: 1 and 24; 25 and 26; 27 and 28; and 11 and 12) were used as PCR primers (see FIG 2b). The first three PCR products were brought to annealing reaction. Then, the DNA fragment containing the three PCR products was employed as a template of recombinant PCR wherein two primers described by SEQ ID NO: 1 and 28 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12.

The final 1431-bp PCR product, HKR127HC(III), encoding the heavy chain of a humanized antibody

(HZKR127III) was introduced into pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(III).

5 In a further embodiment, HKR127KcV(HZII) gene encoding a humanized V<sub>L</sub> region was prepared by grafting the CDR1, CDR3 and partial CDR2 of mouse KR127 light chain to the human DPK12 gene (see FIG 3).

10 However, antibody expressed by using the HKR127KcV(HZII) gene did not show any significant level of binding capacity to corresponding antigen. To improve the binding capacity of HKR127KcV(HZII), we also prepared HKR127KcV(HZI) gene which contains more mouse-derived amino acid residues (see FIG 3) than  
15 HKR127HKcV(HZII) (see FIG 3).

To construct HKR127KC(I) gene encoding a full-length light chain of the humanized antibody of this invention, PCRs were conducted, in which template for amplification is either the HKR127KcV(HZII) gene or  
20 pKC-dfhr-HuS (KCTC 0230BP) containing the light chain leader sequence and the constant region sequence of human immunoglobulin light chain  $\kappa$ .

Three pairs of oligonucleotides (SEQ ID NO: 13 and 14; 15 and 16; and 17 and 18) were used as PCR primers  
25 (see FIG 4).

The first two PCR products were brought to annealing reaction. Then, the DNA fragment containing

the two PCR products was employed as a template of recombinant PCR wherein two primers described by SEQ ID NO: 13 and 16 were used. Another recombinant PCR was conducted to link the amplified 360-bp DNA fragment to DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 17 and 18). The recombinant PCR employed two primers which are described by SEQ ID NO: 13 and 18. The final 739-bp PCR product, HKR127KC(I), encoding the light chain of a humanized antibody (HZKR127I) was introduced into pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127KC(I).

The primers are described in SEQ ID NO: 13 to 18 in SEQUENCE LISTING, and particularly, primer described by SEQ ID NO: 13 contains HindIII sequence at the 5' end, while primer described by SEQ ID NO: 18 does SalI sequence at the 3' end.

The variable region FR of the HKR127KC gene contains 5 mouse KR127-derived codons (see FIG 3). The light chain has 83 FR residues, and the unmodified FR residues is 78. Thus, the amino acid sequence of the light chain variable FR of the HKR127KC gene is 94% identical to that of human DP7 gene.

In addition, this invention provides expression vectors containing genes encoding the humanized  $V_H$  or  $V_L$  region and provides *E. coli* transformants containing

said expression vector.

In other preferred embodiments, expression vectors are prepared, which contain the gene encoding the heavy or light chain of humanized antibody (see FIG 5a, 5b or 5c).

Particularly, two kinds of DNA fragment corresponding to humanized heavy chain was respectively obtained from the plasmids pHKR127HC(I) and pHKR127HC(III) by treatment of restriction enzymes, and then inserted into pRc/CMV (Invitrogen) to give expression vector pCMV-HKR127HC (see FIG5a) and pCMV-HKR127(III)HC (see FIG 5c), respectively.

In addition, DNA fragment encoding the humanized light chain was isolated from the pHKR127KC vector, and then introduced into pCMV-dhfr (KCTC 8671P) to construct expression vector pKC-dhfr-HKR127 (see FIG 5b).

*E. coli* strain DH5 $\alpha$  was transformed with the expression vector pCMV-HKR127HC, pCMV-HKR127(III)HC or pKC-dhfr-HKR127. The resulting *E. coli* transformants containing pCMV-HKR127HC or pKC-dhfr-HKR127 were deposited in KCTC (Korean Collection for Type Culture) (Accession Number: KCTC 0531BP and KCTC 0529BP, respectively) on October 12, 1998. The *E. coli* transformant containing pCMV-HKR127(III)HC was deposited in KCTC (Accession Number: KCTC 0691BP, respectively) on November 15, 1999.

In another preferred embodiment, humanized antibodies specific for HBV surface antigen pre-S1 were expressed in animal cells and obtained from culture media of the cells. COS7 cells were transiently cotransfected with the expression vectors pCMV-HKR127HC and pKC-dhfr-HKR127, and the resulting transfected cells was cultured and the culture supernatant was used to characterize a humanized antibody HZKR127I of the present invention. COS7 cells were also cotransfected with the expression vectors pCMV-HKR127(III)HC and pKC-dhfr-HKR127, and the culture supernatant of transfected cells was used to characterize a humanized antibody HZKR127III.

This invention further provides pharmaceutical compositions containing said humanized antibody.

According to still other preferred embodiments, it was verified that HZKR127I and HZKR127III humanized antibodies of the present invention, showed almost same antigen-binding affinity when compared with mouse monoclonal antibody KR127 (see Table 1, 2 and FIG 6a, 6b).

The composition includes a therapeutically effective amounts of the humanized antibody against HBV antigen pre-S1, with/without a pharmaceutically acceptable delivery vehicle. Moreover, the

compositions may include other anti-hepatitis drug(s), such as anti-S monoclonal antibody or lamivudin.

The humanized antibody against HBV antigen pre-S1 may be formulated with a pharmaceutical vehicle or diluent for intravenous, subcutaneous, intramuscular administration. The pharmaceutical composition can be formulated in a classical manner using solid or liquid vehicles, diluents and additives appropriate to the desired mode of administration.

The humanized antibody of this invention may be administered in a dosage range of about 1 ~ 10 mg/kg, preferably 3 ~ 5 mg/kg, and may be administered once a week.

#### EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of gene encoding humanized heavy chain

In order to construct the humanized heavy chain variable region gene, first, we selected a human immunoglobulin heavy chain gene that shows the highest homology of amino acid sequence to the heavy chain variable region of the mouse monoclonal antibody KR127. As the result, a human immunoglobulin germ line gene DP7 was selected from GenBank database. Then, we constructed a humanized V<sub>H</sub> region gene HKR127HCv(HZII) by DNA recombination techniques, which was based upon the comparison of the mouse KR127 V<sub>H</sub> region with the human DP7 V<sub>H</sub> region. Since the humanized heavy chain did not show significant antigen binding activity, we prepared HKR127HCv(HZI) gene encoding another V<sub>H</sub> region in order to improve the HKR127HCv(HZII) gene (see FIG 1).

Particularly, the HKR127HCv(HZII) gene was constructed by grafting the V<sub>H</sub> region of human DP7 gene with the partial CDR1, 2, and 3 and one FR residue at position 72 of mouse KR127 V<sub>H</sub> region. It was assumed that the human CDRs and FR amino acid residues affected the antigen-binding affinity of the antibody.

Therefore, HKR127HCv(HZI) gene was constructed by PCR employing HKR127HCv(HZII) gene as a template.



On the other hand, a vector pRc/CMV-HC-HuS (Accession Number: KCTC 0229BP) was used to synthesize DNA sequence encoding human C<sub>H</sub> region as well as heavy chain leader sequence, which is required in proper secretion of the heavy chain.

Finally, HKR127HC(I) gene encoding a humanized heavy chain was constructed by recombinant PCR for the annealing of the heavy chain leader sequence, HKR127HCv(HZI) gene, and the human C<sub>H</sub> gene (see FIG 2a).

The primers in these PCRs are synthetic oligonucleotides described by SEQ ID NO: 1 to 12. PCR was performed by using Taq DNA polymerase, and its thermocycle was repeated 30 times, consisting of 1 minute at 94°C, 1 minute at 55°C, and then 1 minute at 72°C. Five pairs of oligonucleotides (SEQ ID NO: 1 and 2; 3 and 4; 5 and 6; 7 and 8; and 9 and 10) were used as PCR primers, and the five PCR products (113 bp; 96 bp; 120 bp; 78 bp; and 87 bp, respectively) were brought to annealing reaction. Then, the DNA fragments containing the five PCR products were employed as template of the recombinant PCR wherein primers described by SEQ ID NO: 1 and 10 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to 1015-bp DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12.

The final PCR product (HKR127HC(I), about 1431-bp) encoding a recombinant heavy chain of humanized antibody was introduced into the EcoRI-SalI site of pBluescript SK(+) vector (Clontech), and the resulting  
5 vector was designated pHKR127HC(I). The DNA sequence of the inserted gene was determined by dideoxynucleotide method.

To more humanize the HKR127HC(I), another humanized heavy chain gene, HKR127(III), which has much  
10 less number of mouse FR residues, was constructed.

To construct the HKR127HC(III), HKR127HCv(HZIII) gene was constructed by PCR employing HKR127HCv(HZII) gene as a template. On the other hand, a vector pRc/CMV-HC-HuS (Accession Number: KCTC 0229BP) was used  
15 to synthesize DNA sequence encoding human C<sub>H</sub> region as well as heavy chain leader sequence, which is required in proper secretion of the heavy chain.

Finally, HKR127HC(III) gene encoding a humanized heavy chain was constructed by recombinant PCR for  
20 annealing of the heavy chain leader sequence, HKR127HCv(HZIII) gene, and the human C<sub>H</sub> gene (see FIG 2b).

The primers in these PCRs are synthetic oligonucleotides described by SEQ ID NO: 24 to 28. PCR  
25 was performed by using Taq DNA polymerase, and its thermocycle was repeated 30 times, consisting of 1 minute at 94°C, 1 minute at 55°C, and then 1 minute at

72°C. Three pairs of oligonucleotides (SEQ ID NO: 1 and 24; 25 and 26; and 27 and 28) were used as PCR primers, and the three PCR products (179 bp; 141 bp; and 87 bp, respectively) were brought to annealing reaction. Then, the DNA fragments containing the three PCR products were employed as template of the recombinant PCR wherein primers described by SEQ ID NO: 1 and 28 were used. Another recombinant PCR was conducted to link the amplified 431-bp DNA fragment to 1015-bp DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 11 and 12). The recombinant PCR employed two primers described by SEQ ID NO: 1 and 12.

The final PCR product (HKR127HC(III)), about 1431-bp) encoding a recombinant heavy chain of humanized antibody was introduced into the EcoRI-SalI site of pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127HC(III). The DNA sequence of the inserted gene was determined by dideoxynucleotide method.

#### Example 2: Preparation of gene encoding humanized light chain

In order to prepare humanized light chain containing variable region, we devised genes encoding

the light chain. First, we selected a human  $\kappa$  immunoglobulin gene that shows the highest homology of amino acid sequence to the light chain of the mouse monoclonal antibody KR127. As the result, a human  $\kappa$  immunoglobulin gene DPK12 was selected from GenBank database. Then, we constructed HKR127KcV(HZII) gene encoding a humanized  $V_L$  region by grafting CDR1, partial CDR2, and CDR3 and one FR residue at position 41 of the mouse KR127  $V_L$  region to the human DPK12  $V_L$  region. The resulting humanized  $V_L$  was not functional in antigen-binding. To improve the HKR127KcV(HZII) gene, we constructed HKR127KcV(HZI) gene encoding another  $V_L$  region (see FIG 3).

The HKR127KcV(HZI) gene was constructed by grafting the  $V_L$  region of human DPK12 antibody with a few FR residues and CDR1, CDR2 and CDR3 of mouse KR127  $V_L$  (see FIG 3).

On the other hand, a vector pKC-dhfr-HuS (Accession Number: KCTC 0230BP) was used to synthesize DNA sequence encoding human  $C_L$  region as well as light chain leader sequence, which is required in proper secretion of the light chain.

Finally, HKR127Kc(I) gene encoding a humanized light chain was prepared by recombinant PCR for the annealing of the PCR products, light chain leader sequence, the HKR127KcV(HZI) gene, and the human  $C_L$  gene (see FIG 4).

The primers in these PCRs are synthetic oligonucleotides described by SEQ ID NO: 13 to 18. The thermocycle of these PCRs was repeated 30 times, consisting of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C. Two pairs of oligonucleotides (SEQ ID NO: 13 and 14; and SEQ ID NO: 15 and 16) were used as PCR primers, and the two PCR products (101 bp and 159 bp, respectively) were brought to annealing reaction. Then, the DNA fragments containing the two PCR products was employed as a template of recombinant PCR wherein primers described by SEQ ID NO: 13 and 16 were used. Another recombinant PCR was conducted to link the amplified 248-bp DNA fragment to 515-bp DNA fragment which was obtained by PCR using two primers (described by SEQ ID NO: 17 and 18). The recombinant PCR employed two primers described by SEQ ID NO: 13 and 18.

The final PCR product (HKR127KC(I), 736-bp) encoding a recombinant light chain of humanized antibody was introduced into the HindIII-SalI site of pBluescript SK(+) vector (Clontech), and the resulting vector was designated pHKR127KC(I). The DNA sequence of the inserted gene was determined by dideoxynucleotide method.

Example 3: Construction of expression vector containing the humanized heavy chain gene

The pHKR127HC(I) or pHKR127HC(III) plasmid of Example 1 was digested with SalI enzyme, and the both ends of the vector was made blunt using Klenow enzyme treatment. This DNA fragment was further digested with NotI enzyme to obtain the gene encoding humanized heavy chain.

On the other hand, pRc/CMV (Invitrogen) was cut with XbaI enzyme, and the ends of the vector was made blunt by treating with Klenow enzyme, and then digested with NotI.

The humanized heavy chain gene and the linearized vector were linked to give expression vector pCMV-HKR127HC or pCMV-HKR127(III)HC. The *E. coli* transformant containing pCMV-HKR127HC or pCMV-HKR127(III)HC was deposited in KCTC (Korean Collection for Type Culture) (Accession Number: KCTC 0531BP and KCTC 0691BP, respectively), and the expression vector pCMV-HKR127HC and pCMV-HKR127(III)HC is shown in FIG 5a and 5c, respectively.

Example 4: Construction of expression vector containing  
the humanized light chain gene

The pHKR127KC vector of Example 2 was digested with HindIII and ApaI enzymes, and the resulting  
5 fragment was inserted into HindIII-ApaI site of pCMV-dhfr (Accession Number: KCTC 8671P) to give expression vector pKC-dhfr-HKR127. The *E. coli* transformant containing pKC-dhfr-HKR127 was deposited in KCTC (Korean Collection for Type Culture) (Accession Number:  
10 KCTC 0529BP), and the expression vector pKC-dhfr-HKR127 is shown in FIG 5b.

Example 5: Expression of humanized antibody in COS7  
cells

15 COS7 cells were maintained in DMEM (Gibco) supplemented by 10% calf serum at 37°C, under 5% CO<sub>2</sub> condition. The cells were inoculated in 100mm petri dishes, and then incubated at 37°C overnight.

To express a humanized antibody HZKR127I, 5 µg of  
20 pCMV-HKR127HC or pKC-dhfr-HKR127 was diluted with 800 µl of OPTI MEM I (Gibco), and 50 µl of Lipofectamin (Gibco) was also diluted with 800 µl of OPTI MEM I. These mixtures in 15-ml tubes were incubated at room temperature for 15 minutes or more. In the meantime,

COS7 cells were washed twice with OPTI MEM I.

OPTI MEM I (6.4 ml) was added to the DNA-Lipofectamin mixture, mixed well, and poured on the COS7 cells. After the cells were cultured in a CO<sub>2</sub> incubator for 72 hours, the medium was centrifuged, and the supernatant was concentrated by ultrafiltration kit. The concentration of antibody was determined by Sandwich ELISA using anti-human IgG and anti-human IgG-HRP (horseradish peroxidase) conjugate.

To express and obtain a humanized antibody HZKR127III, the same protocol was repeated except using pCMV-HKR127(III)HC instead of using pCMV-HKR127HC.

Example 6: Binding activity of humanized antibody to HBV surface antigen pre-S1

We prepared HBV surface antigen pre-S1 (amino acid residue 1-56; Kim and Hong, Biotechnology Letters, 17:871-876, 1995) and 1 µg of the purified pre-S1 was coated on each well in microplates. After addition of 0, 0.25, 0.5, 1, 2, 3, 4, 5, 7.5, 10, 20, or 40 ng of the humanized antibodies prepared in Example 5, indirect ELISA was performed, in which secondary antibody was Fc-specific anti-human IgG-HRP conjugate. The binding activities of the antibodies were determined by measuring OD at 492 nm.



Purified mouse KR127 antibody was used as a control, and ELISA of KR127 antibody was conducted using Fc-specific anti-mouse IgG-HRP conjugate as a secondary antibody. The result is presented in Table 1 and 2.

Table 1.

Binding activity of KR127 and HZKR127I to HBV surface antigen pre-S1 (OD at 492 nm)

| Amount<br>(ng)<br>Antibody | 0    | 0.25 | 0.5  | 1    | 2    | 3    | 4    | 5    | 7.5  | 10   | 20   | 40   |
|----------------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| KR127                      | 0.09 | 0.12 | 0.15 | 0.20 | 0.30 | 0.36 | 0.43 | 0.54 | 0.60 | 0.80 | 1.16 | 1.64 |
| HZKR127I                   | 0.09 | 0.12 | 0.17 | 0.26 | 0.35 | 0.43 | 0.60 | 0.71 | 0.79 | 1.12 | 1.48 | 1.77 |

Table 2.

Binding activity of HZKR127I and HZKR127III to HBV surface antigen pre-S1 (OD at 492 nm)

| Amount<br>(ng)<br>Antibody | 0    | 0.25 | 0.5  | 1    | 2    | 3    | 4    | 5    | 7.5  | 10   | 20   | 40   |
|----------------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| HZKR127I                   | 0.06 | 0.19 | 0.25 | 0.58 | 0.65 | 0.75 | 0.86 | 1.02 | 1.25 | 1.39 | 1.95 | 2.07 |
| HZKR127III                 | 0.06 | 0.20 | 0.37 | 0.60 | 0.87 | 1.10 | 1.24 | 1.37 | 1.65 | 1.89 | 2.04 | 2.10 |

Example 7: Antigen-binding affinity of humanized antibody to HBV surface antigen pre-S1

Antigen-binding affinity to HBV surface antigen

pre-S1 was assayed by competitive ELISA method(Ryu et al., J. Med. Virol., 52:226, 1997).

Binding reactions between the pre-S1 antigen ( $1 \times 10^{-7} \sim 1 \times 10^{-12}$  M) and the humanized antibody of Example 5 (5 ng), or between the antigen ( $1 \times 10^{-7} \sim 1 \times 10^{-12}$  M) and control antibody KR127 (5 ng), were performed at 37°C for 2 hours. Then the reaction mixtures were added to 96-well microplates coated with the 250 ng of antigen pre-S1.

FIG 6a shows the affinity of two kinds of antibodies. It was confirmed that the binding affinity of the humanized antibody HZKR127I is almost same as that of the mouse antibody KR127 ( $7 \times 10^7$  M<sup>-1</sup>).

FIG 6b shows the affinity of HZKR127III compared with that of HZKR127I. The affinity of HZKR127III ( $5 \times 10^7$  M<sup>-1</sup>) was not much different from that ( $7 \times 10^7$  M<sup>-1</sup>) of HZKR127I.

#### INDUSTRIAL APPLICABILITY

As shown above, the present invention provides humanized antibody against HBV surface antigen pre-S1, which shows similar level of binding affinity when compared with mouse monoclonal antibody, whereas immunogenicity of the humanized antibody is remarkably reduced. Thus, the humanized antibody of the present

invention may be useful for the prevention of HBV infection and for the treatment of hepatitis B.

Those skilled in the art will appreciate that the  
5 conceptions and specific embodiments disclosed in the  
foregoing description may be readily utilized as a  
basis for modifying or designing other embodiments for  
carrying out the same purposes of the present invention.  
Those skilled in the art will also appreciate that such  
10 equivalent embodiments do not depart from the spirit  
and scope of the invention as set forth in the appended  
claims.


BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT  
OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

## INTERNATIONAL FORM

## RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO: Hong Hyo-Jeong  
KIT Apt. 15-401, #237 Gajeong-dong, Yusong-ku, Taejon 305-350,  
Republic of Korea

|   |   |
|---|---|
| <b>I. IDENTIFICATION OF THE MICROORGANISM</b>   |   |
| Identification reference given by the<br>DEPOSITOR:<br><br><b><i>Escherichia coli</i> DH5<math>\alpha</math></b><br><b>/pCMV-HKR127HC</b>   | Accession number given by the<br>INTERNATIONAL DEPOSITARY<br>AUTHORITY:<br><br><b>KCTC 0531BP</b>   |
| <b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>   |   |
| The microorganism identified under I above was accompanied by:<br><input checked="" type="checkbox"/> a scientific description<br><input type="checkbox"/> a proposed taxonomic designation<br>(Mark with a cross where applicable) |   |
| <b>III. RECEIPT AND ACCEPTANCE</b>  |   |
| This International Depositary Authority accepts the microorganism identified under I above,<br>which was received by it on <b>October 12 1998</b>   |   |
| <b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>  |   |
| The microorganism identified under I above was received by this International Depositary<br>Authority on _____ and a request to convert the original deposit to a deposit<br>under the Budapest Treaty was received by it on _____. |   |
| <b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>  |   |
| Name: <b>Korean Collection for Type Cultures</b><br><br>Address: <b>Korea Research Institute of<br/>Bioscience and Biotechnology<br/>(KRIBB)</b><br><b>#52, Oun-dong, Yusong-ku,<br/>Taejon 305-333,<br/>Republic of Korea</b>      | Signature(s) of person(s) having the power to<br>represent the International Depositary<br>Authority or of authorized official(s):<br><br><br><b>PARK Yong-Ha, Director</b><br><b>Date: October 17 1998</b> |


BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT  
OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

## INTERNATIONAL FORM

## RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

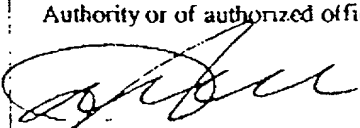
issued pursuant to Rule 7.1

TO: HONG, Hyo Jeong  
KIT Apt. 15 401, #Z37, Kajeong-dong, Yusong-ku, Taejeon 305-350,  
Republic of Korea

|   |   |
|---|---|
| <b>I. IDENTIFICATION OF THE MICROORGANISM</b>   |   |
| Identification reference given by the<br>DEPOSITOR:<br><br><i>Escherichia coli</i><br>DH5 $\alpha$ /pCMV-HKR127(III)HC  | Accession number given by the<br>INTERNATIONAL DEPOSITARY<br>AUTHORITY:<br><br>KCTC 0691BP  |
| <b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>   |   |
| The microorganism identified under I above was accompanied by:<br><input checked="" type="checkbox"/> a scientific description<br><input type="checkbox"/> a proposed taxonomic designation<br>(Mark with a cross where applicable) |   |
| <b>III. RECEIPT AND ACCEPTANCE</b>  |   |
| This International Depositary Authority accepts the microorganism identified under I above,<br>which was received by it on <b>November 15 1999</b> .  |   |
| <b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>  |   |
| The microorganism identified under I above was received by this International Depositary<br>Authority on _____ and a request to convert the original deposit to a deposit<br>under the Budapest Treaty was received by it on _____. |   |
| <b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>  |   |
| Name: Korean Collection for Type Cultures<br><br>Address: Korea Research Institute of<br>Bioscience and Biotechnology<br>(KRIBB)<br>#52, Oun-dong, Yusong-ku,<br>Taejeon 305-333,<br>Republic of Korea                              | Signature(s) of person(s) having the power<br>to represent the International Depositary<br>Authority of authorized official(s):<br><br>BAE, Kyung Sook, Director<br>Date: <b>November 18 1999</b> |

INTERNATIONAL FORM  
**RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT**  
 issued pursuant to Rule 7.1

TO: Hong Hyo-Jeong  
 KIT Apt. 15-401, #237 Gajeong-dong, Yusong-ku, Taejon 305-350,  
 Republic of Korea

|  |   |
|--|---|
| <b>I. IDENTIFICATION OF THE MICROORGANISM</b>  |   |
| Identification reference given by the DEPOSITOR:<br><br><b><i>Escherichia coli</i> DH5@<br/>         /pKC-dhfr-BKR127</b>  | Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:<br><br><b>KCTC 0529BP</b>   |
| <b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>  |   |
| The microorganism identified under I above was accompanied by:<br><input checked="" type="checkbox"/> a scientific description<br><input type="checkbox"/> a proposed taxonomic designation<br>(Mark with a cross where applicable)                      |   |
| <b>III. RECEIPT AND ACCEPTANCE</b>   |   |
| This International Depositary Authority accepts the microorganism identified under I above, which was received by it on <b>October 12 1998</b>   |   |
| <b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>   |   |
| The microorganism identified under I above was received by this International Depositary Authority on _____ and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____                             |   |
| <b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>   |   |
| Name: <b>Korean Collection for Type Cultures</b><br><br>Address: <b>Korea Research Institute of<br/>         Bioscience and Biotechnology (KRIBB)<br/>         #52, Oun-dong, Yusong-ku,<br/>         Taejon 305-333,<br/>         Republic of Korea</b> | Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):<br><br><br><b>PARK Yong-Ha, Director</b><br>Date: <b>October 17 1998</b> |

What is Claimed is

1. (deleted)

2. (amended) A humanized antibody specific for HBV surface antigen pre-S1, containing humanized heavy chain variable region which comprises amino acid sequence described by SEQ ID NO: 20.

3. (amended) A humanized antibody specific for HBV surface antigen pre-S1, containing humanized heavy chain variable region which comprises amino acid sequence described by SEQ ID NO: 21.

4. (amended) The humanized antibody of claim 3, wherein the humanized heavy chain variable region comprises an amino acid sequence which is modified from an amino acid residue of SEQ ID NO: 21 by at least one amino acid substitution selected from the group comprising

Lys<sup>28</sup> → Val<sup>28</sup>,                      Thr<sup>29</sup> → Ala<sup>29</sup>,                      Thr<sup>30</sup> → Ser<sup>30</sup>,  
 Met<sup>31</sup> → Ile<sup>31</sup>,                      Arg<sup>67</sup> → Lys<sup>67</sup>,                      Val<sup>68</sup> → Ala<sup>68</sup>,  
 Leu<sup>69</sup> → Ile<sup>69</sup>,                      Val<sup>70</sup> → Ala<sup>70</sup>, and Tyr<sup>75</sup> → Phe<sup>75</sup>.

17. 01. 2001

5. (amended) A humanized antibody specific for HBV surface antigen pre-S1, containing humanized light chain variable region comprises amino acid sequence described by SEQ ID NO: 23.

6. A gene encoding humanized heavy chain which contains a humanized heavy chain variable region of claim 2, 3 or 4.

7. The gene of claim 6, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 20.

8. The gene of claim 6, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 21.

9. A gene encoding humanized light chain which contains a humanized light chain variable region comprising amino acid sequence described by SEQ ID NO: 23.

10. An expression vector containing the gene of claim 6.

11. The expression vector of claim 10, pCMV-HKR127HC, wherein the gene of claim 7 is inserted into pRC/CMV

AMENDED SHEET



(Accession Number: KCTC 0531BP).

12. The expression vector of claim 10, pCMV-HKR127(III)HC, wherein the gene of claim 8 is inserted into pRC/CMV (Accession Number: KCTC 0691BP).

13. An expression vector containing the gene of claim 9.

14. The expression vector of claim 13, pKC-dhfr-HKR127, wherein the gene of claim 9 is inserted into pCMV-dhfr (Accession Number: KCTC 0529BP).

15. **(amended)** Pharmaceutical composition containing the humanized antibody of claim 2,3,4 or 5, which may be administered in order to prevent HBV infection or to treat chronic hepatitis B.

AMENDED SHEET

FIG. 1a

|         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|         | Q   | V   | Q   | L   | Q   | Q   | S   | G   | P   | E   | L   | V   | K   | P   |     |
| KR127VH | CAG | GTC | CAG | CTG | CAG | CAG | TCT | GGA | CCT | GAA | CTG | GTG | AAG | CCT | 42  |
| DP7     | CAG | GTG | CAG | CTG | GTG | CAG | TCT | GGG | GCT | GAG | GTG | AAG | AAG | CCT |     |
| HZII    | CAG | GTC | CAG | CTG | GTG | CAG | TCT | GGA | GCT | GAA | GTG | AAG | AAG | CCT |     |
| HZI     | CAG | GTC | CAG | CTG | GTG | CAG | TCT | GGA | GCT | GAA | GTG | GTG | AAG | CCT |     |
| HZIII   | CAG | GTC | CAG | CTG | GTG | CAG | TCT | GGA | GCT | GAA | GTG | AAG | AAG | CCT | 42  |
| HZII    | -   | -   | -   | -   | V   | -   | -   | -   | A   | -   | V   | K   | -   | -   |     |
| HZI     | -   | -   | -   | -   | V   | -   | -   | -   | A   | -   | V   | -   | -   | -   |     |
| HZIII   | -   | -   | -   | -   | V   | -   | -   | -   | A   | -   | V   | K   | -   | -   |     |
|         | G   | A   | S   | V   | K   | I   | S   | C   | K   | A   | S   | G   | Y   | A   |     |
| KR127VH | GGG | GCC | TCA | GTG | AAG | ATT | TCC | TGC | AAA | GCT | TCT | GGC | TAC | GCA | 84  |
| DP7     | GGG | GCC | TCA | GTG | AAG | GTT | TCC | TGC | AAG | GCA | TCT | GGA | TAC | ACC |     |
| HZII    | GGG | GCC | TCA | GTG | AAG | GTT | TCC | TGC | AAA | GCT | TCT | GGC | TAC | ACC |     |
| HZI     | GGG | GCC | TCA | GTG | AAG | GTT | TCC | TGC | AAA | GCT | TCT | GGC | TAC | GCA |     |
| HZIII   | GGG | GCC | TCA | GTG | AAG | GTT | TCC | TGC | AAA | GCT | TCT | GGC | TAC | ACC | 84  |
| HZII    | -   | -   | -   | -   | -   | V   | -   | -   | -   | -   | -   | -   | -   | T   |     |
| HZI     | -   | -   | -   | -   | -   | V   | -   | -   | -   | -   | -   | -   | -   | -   |     |
| HZIII   | -   | -   | -   | -   | -   | V   | -   | -   | -   | -   | -   | -   | -   | T   |     |
|         | F   | S   | S   | S   | W   | M   | N   | W   | V   | K   | Q   | R   | P   | G   |     |
| KR127VH | TTC | AGT | AGT | TCT | TGG | ATG | AAC | TGG | GTG | AAG | CAG | AGG | CCT | GGA | 126 |
| DP7     | TTC | ACC | AGC | TAC | TAT | ATG | CAC | TGG | GTG | CGA | CAG | GCC | CCT | GGA |     |
| HZII    | TTC | ACC | AGT | TAC | TGG | ATG | AAC | TGG | GTG | CGA | CAG | GCC | CCT | GGA |     |
| HZI     | TTC | AGT | AGT | TCT | TGG | ATG | AAC | TGG | GTG | CGA | CAG | GCC | CCT | GGA |     |
| HZIII   | TTC | ACC | AGT | TCT | TGG | ATG | AAC | TGG | GTG | CGA | CAG | GCC | CCT | GGA |     |
| HZII    | -   | T   | -   | Y   | -   | -   | -   | -   | -   | R   | -   | A   | -   | -   |     |
| HZI     | -   | -   | -   | -   | -   | -   | -   | -   | -   | R   | -   | A   | -   | -   |     |
| HZIII   | -   | T   | -   | -   | -   | -   | -   | -   | -   | R   | -   | A   | -   | -   |     |
|         | Q   | G   | L   | E   | W   | I   | G   | R   | I   | Y   | P   | G   | D   | G   |     |
| KR127VH | CAG | GGT | CTT | GAG | TGG | ATT | GGA | CGG | ATT | TAT | CCT | GGA | GAT | GGA | 168 |
| DP7     | CAA | GGG | CTT | GAG | TGG | ATG | GGA | ATA | ATC | AAC | CCT | AGT | GGT | GGT |     |
| HZII    | CAG | GGT | CTT | GAG | TGG | ATG | GGA | CGG | ATT | TAT | CCT | GGA | GAT | GGA |     |
| HZI     | CAG | GGT | CTT | GAG | TGG | ATT | GGA | CGG | ATT | TAT | CCT | GGA | GAT | GGA |     |
| HZIII   | CAG | GGT | CTT | GAG | TGG | ATG | GGA | CGG | ATT | TAT | CCT | GGA | GAT | GGA |     |
| HZII    | -   | -   | -   | -   | -   | M   | -   | -   | -   | -   | -   | -   | -   | -   |     |
| HZI     | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |     |
| HZIII   | -   | -   | -   | -   | -   | M   | -   | -   | -   | -   | -   | -   | -   | -   |     |
|         | D   | T   | N   | Y   | N   | G   | K   | F   | K   | G   | K   | A   | T   | L   |     |
| KR127VH | GAT | ACT | AAC | TAC | AAT | GGG | AAG | TTC | AAG | GGC | AAG | GCC | ACA | CTG | 210 |
| DP7     | AGC | ACA | AGC | TAC | GCA | CAG | AAG | TTC | CAG | GGC | AGA | GTC | ACC | ATG |     |
| HZII    | GAT | ACT | AAC | TAC | GCA | CAG | AAG | TTC | CAG | GGC | AGA | GTC | ACA | ATG |     |
| HZI     | GAT | ACT | AAC | TAC | GCA | CAG | AAG | TTC | CAG | GGC | AAG | GCC | ACA | CTG |     |
| HZIII   | GAT | ACT | AAC | TAC | GCA | CAG | AAG | TTC | CAG | GGC | AGA | GTC | ACA | ATG |     |
| HZII    | -   | -   | -   | -   | A   | Q   | -   | -   | Q   | -   | R   | V   | -   | M   |     |
| HZI     | -   | -   | -   | -   | A   | Q   | -   | -   | Q   | -   | -   | -   | -   | -   |     |
| HZIII   | -   | -   | -   | -   | A   | Q   | -   | -   | Q   | -   | R   | V   | -   | M   |     |

FIG. 1b

KR127VH T A D K S S S T A Y M Q L S 252  
 DP7 ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC AGC  
 HZII ACT GCA GAC ACG TCC ACG AGC ACA GTC TAC ATG GAG CTC AGC  
 HZI ACC GCA GAC AAA TCC ACG AGC ACA GCC TAC ATG GAG CTC AGC  
 HZIII ACT GCA GAC AAA TCC ACG AGC ACA GTC TAC ATG GAG CTC AGC  
 HZII - - - T - T - - V - - E - -  
 HZI - - - - - T - - - - - E - -  
 HZIII - - - - - T - - - V - - E - -

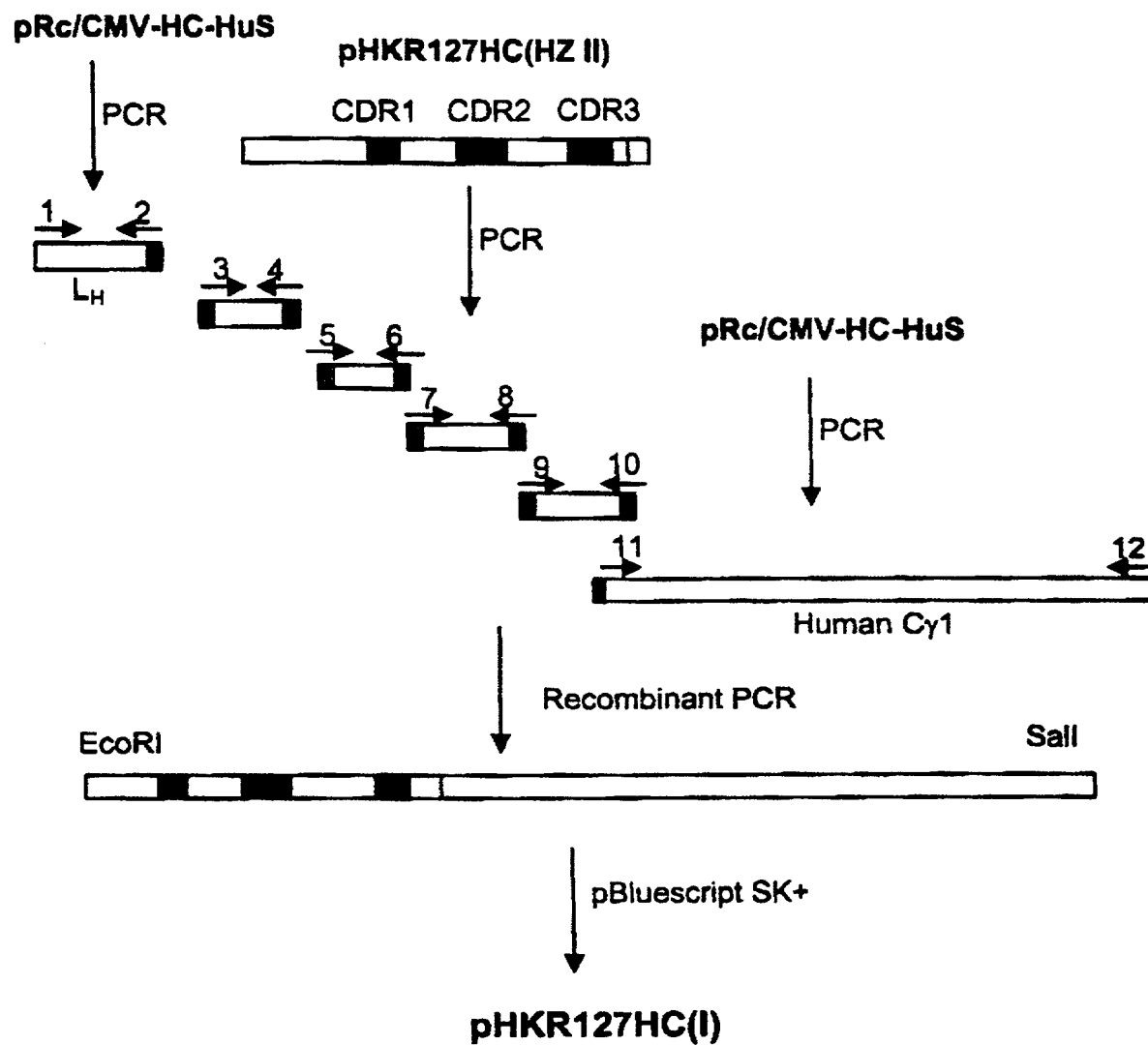
KR127VH S L T S V D S A V Y F C A R 294  
 DP7 AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TTC TGT GCA AGA  
 HZII AGC CTG AGA TCT GAG GAC ACG GCG GTC TAT TAC TGT GCA AGA  
 HZI AGC CTG AGA TCT GAG GAC ACG GCG GTC TAT TTC TGT GCA AGA  
 HZIII AGC CTG AGA TCT GAG GAC ACG GCG GTC TAT TAC TGT GCA AGA  
 HZII - - R - E - T - - - Y - - -  
 HZI - - R - E - T - - - - - - -  
 HZIII - - R - E - T - - - - Y - - -

KR127VH E Y D E A Y W G Q G T L V T 336  
 GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT  
 HZII GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT  
 HZI GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT  
 HZIII GAG TAC GAC GAG GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT  
 HZII - - - - D - - - - - - -  
 HZI - - - - - - - - - - - -  
 HZIII - - - - - - - - - - - -

KR127VH V S A  
 GTC TCT GCA 345

HZII GTC TCT TCA  
 HZI GTC TCT TCA  
 HZIII GTC TCT TCA  
 HZII - - S  
 HZI - - S  
 HZIII - - S

FIG. 2a



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FIG. 2b

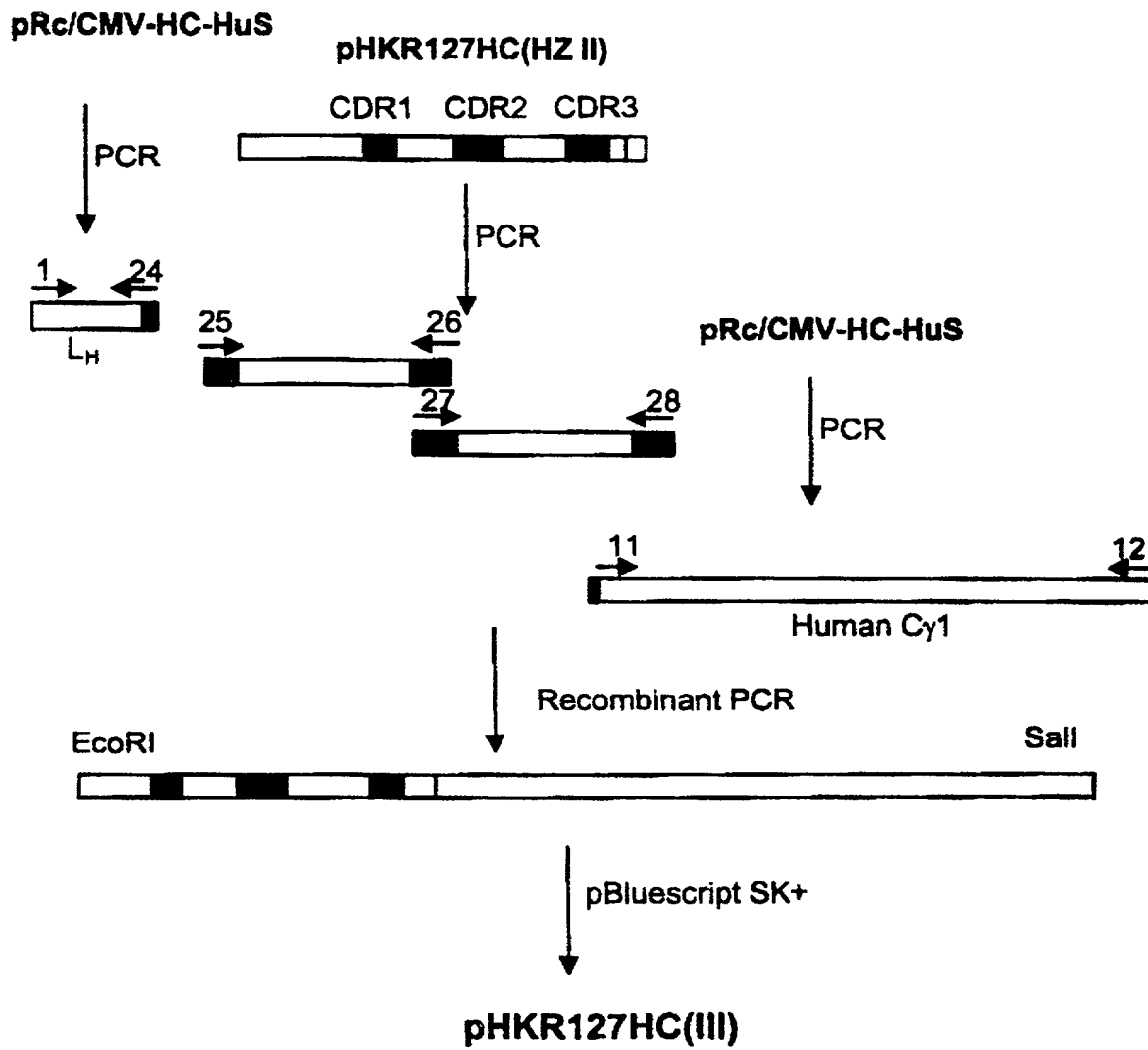


FIG. 3a

|         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|         | D   | I   | L   | M   | T   | Q   | T   | P   | L   | I   | L   | S   | V   | T   |     |
| KR127VK | GAT | ATC | TTG | ATG | ACC | CAA | ACT | CCA | CTT | ATT | TTG | TCG | GTT | ACC | 42  |
| DPK12   | GAT | ATT | GTG | ATG | ACC | CAG | ACT | CCA | CTC | TCT | CTG | TCC | GTC | ACC |     |
| HZII    | GAT | ATC | GTG | ATG | ACC | CAA | ACT | CCA | CTT | TCT | TTG | TCG | GTT | ACC |     |
| HZII    | -   | -   | V   | -   | -   | -   | -   | -   | -   | S   | -   | -   | -   | -   |     |
| HZI     | -   | -   | -   | -   | -   | -   | -   | -   | -   | S   | -   | -   | -   | -   |     |
|         | I   | G   | Q   | P   | A   | S   | I   | S   | C   | K   | S   | S   | Q   | S   |     |
| KR127VK | ATT | GGA | CAA | CCA | GCC | TCT | ATC | TCT | TGC | AAG | TCA | AGT | CAG | AGC | 84  |
| DPK12   | CCT | GGA | CAG | CCG | GCC | TCC | ATC | TCC | TGC | AAG | TCT | AGT | CAG | AGC |     |
| HZII    | CCT | GGA | CAA | CCA | GCC | TCT | ATC | TCT | TGC | AAG | TCA | AGT | CAG | AGC |     |
| HZII    | P   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |     |
| HZI     | P   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |     |
|         | L   | L   | Y   | S   | N   | G   | K   | T   | Y   | L   | N   | W   | L   | L   |     |
| KR127VK | CTC | TTA | TAT | AGT | AAT | GGA | AAA | ACC | TAT | TTG | AAT | TGG | TTA | TTA | 126 |
| DPK12   | CTC | CTG | CAT | AGT | GAT | GGA | AAG | ACC | TAT | TTG | TAT | TGG | TAC | CTG |     |
| HZII    | CTC | TTA | TAT | AGT | AAT | GGA | AAA | ACC | TAT | TTG | AAT | TGG | TTA | TTA |     |
| HZII    | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |     |
| HZI     | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |     |
|         | Q   | R   | P   | G   | Q   | S   | P   | K   | R   | L   | I   | Y   | L   | V   |     |
| KR127VK | CAG | AGG | CCA | GGC | CAG | TCT | CCA | AAG | CGC | CTA | ATC | TAT | CTG | GTG | 168 |
| DPK12   | CAG | AAG | CCA | GGC | CAG | CCT | CCA | CAG | CTC | CTG | ATC | TAT | GAA | GTT |     |
| HZII    | CAG | AAG | CCA | GGC | CAG | CCT | CCA | CAG | CTC | CTA | ATC | TAT | CTG | GTG |     |
| HZII    | -   | K   | -   | -   | -   | P   | -   | Q   | L   | -   | -   | -   | -   | -   |     |
| HZI     | -   | K   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |     |
|         | S   | K   | L   | D   | S   | G   | V   | P   | D   | R   | F   | T   | G   | S   |     |
| KR127VK | TCT | AAA | CTG | GAC | TCT | GGA | GTC | CCT | GAC | AGG | TTC | ACT | GGC | AGT | 210 |
| DPK12   | TCC | AAC | CGG | TTC | TCT | GGA | GTG | CCA | GAT | AGG | TTC | AGT | GGC | AGC |     |
| HZII    | TCT | AAA | CGG | TTC | TCT | GGA | GTC | CCT | GAC | AGG | TTC | AGT | GGC | AGT |     |
| HZII    | -   | -   | R   | F   | -   | -   | -   | -   | -   | -   | -   | S   | -   | -   |     |
| HZI     | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | S   | -   | -   |     |

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FIG. 3b

|         |     |     |     |     |     |     |     |     |     |     |          |     |     |     |     |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------|-----|-----|-----|-----|
|         | G   | S   | G   | T   | D   | F   | T   | L   | K   | I   | I        | R   | V   | E   |     |
| KR127VK | GGA | TCA | GGA | ACA | GAT | TTT | ACA | CTG | AAA | ATC | ATC      | AGA | GTG | GAG | 252 |
| DPK12   | GGG | TCA | GGG | ACA | GAT | TTC | ACA | CTG | AAA | ATC | AGC      | CGG | GTG | GAG |     |
| HZII    | GGA | TCA | GGA | ACA | GAT | TTT | ACA | CTG | AAA | ATC | AGC      | AGA | GTG | GAG |     |
| HZII    | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | <b>S</b> | -   | -   | -   |     |
| HZI     | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | <b>S</b> | -   | -   | -   |     |

|         |     |     |     |            |     |     |     |     |     |                |                |                |                |                |     |
|---------|-----|-----|-----|------------|-----|-----|-----|-----|-----|----------------|----------------|----------------|----------------|----------------|-----|
|         | A   | E   | D   | L          | G   | V   | Y   | Y   | C   | V              | Q              | G              | T              | H              |     |
| KR127VK | GCT | GAG | GAT | TTG        | GGA | GTT | TAT | TAC | TGC | <del>GTG</del> | <del>CAA</del> | <del>GGT</del> | <del>ACA</del> | <del>CAT</del> | 294 |
| DPK12   | GCT | GAG | GAT | GTT        | GGG | GTT | TAT | TAC | TGC | ATG            | CAA            | AGT            | ATA            | CAG            |     |
| HZII    | GCT | GAG | GAT | <b>GTT</b> | GGA | GTT | TAT | TAC | TGC | GTG            | CAA            | GGT            | ACA            | CAT            |     |
| HZII    | -   | -   | -   | <b>V</b>   | -   | -   | -   | -   | -   | -              | -              | -              | -              | -              |     |
| HZI     | -   | -   | -   | <b>V</b>   | -   | -   | -   | -   | -   | -              | -              | -              | -              | -              |     |

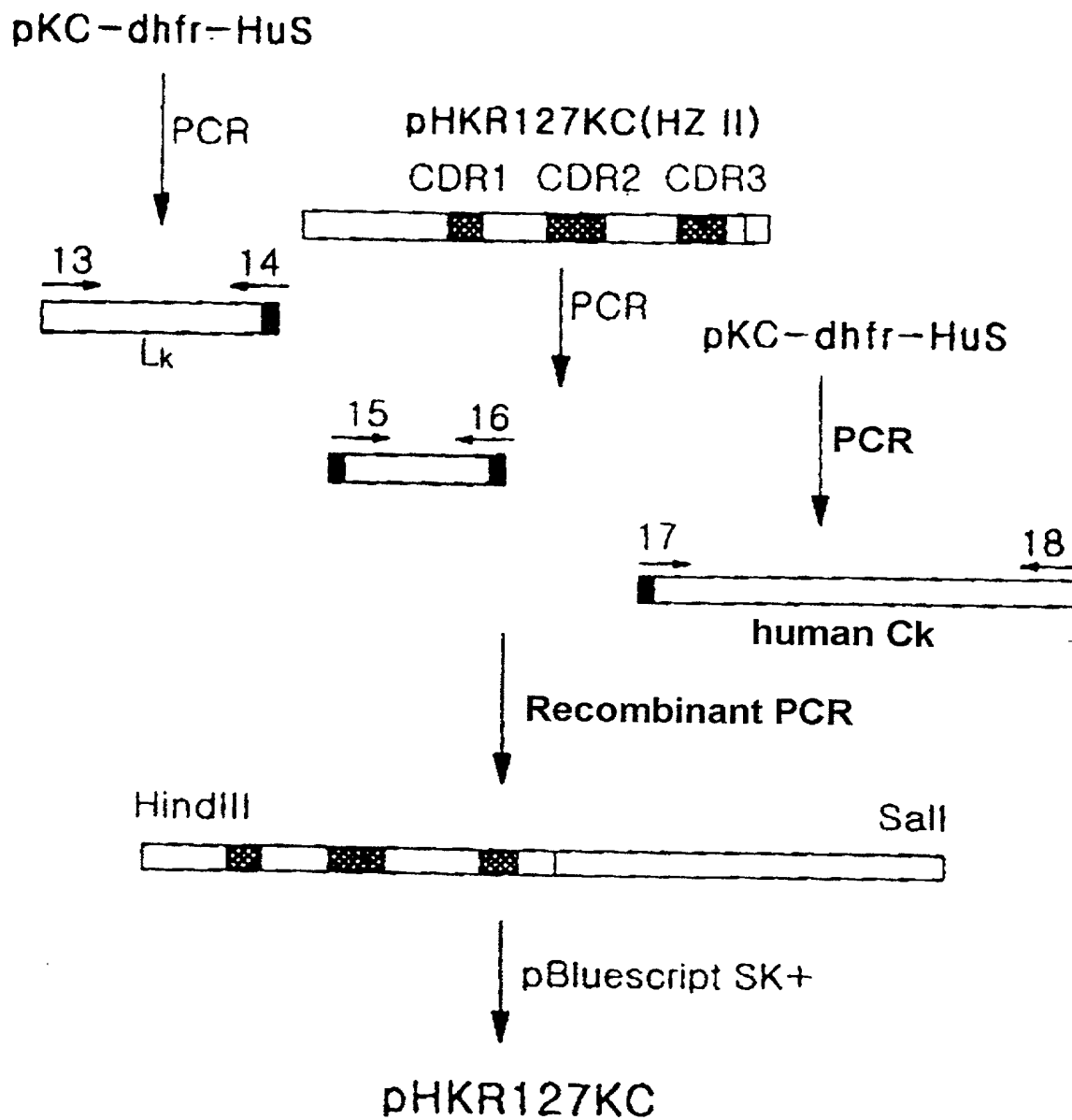
|         |                |                |                |                |     |     |     |     |     |     |            |     |     |     |     |
|---------|----------------|----------------|----------------|----------------|-----|-----|-----|-----|-----|-----|------------|-----|-----|-----|-----|
|         | F              | P              | Q              | T              | F   | G   | G   | G   | T   | K   | L          | E   | I   | K   |     |
| KR127VK | <del>TTT</del> | <del>CCT</del> | <del>CAG</del> | <del>ACG</del> | TTC | GGT | GGA | GGC | ACC | AAG | CTG        | GAA | ATC | AAA | 336 |
| DPK12   | CTT            | CCT            | CC             |                |     |     |     |     |     |     |            |     |     |     |     |
| HZII    | TTT            | CCT            | CAG            | ACG            | TTC | GGT | GGA | GGC | ACC | AAG | <b>GTG</b> | GAA | ATC | AAA |     |
| HZII    | -              | -              | -              | -              | -   | -   | -   | -   | -   | -   | <b>V</b>   | -   | -   | -   |     |
| HZI     | -              | -              | -              | -              | -   | -   | -   | -   | -   | -   | <b>V</b>   | -   | -   | -   |     |

|         |     |     |
|---------|-----|-----|
|         | R   |     |
| KR127VK | CGG | 339 |
| HZII    | CGG |     |
| HZII    | -   |     |
| HZI     | -   |     |

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FIG. 4





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FIG. 5a

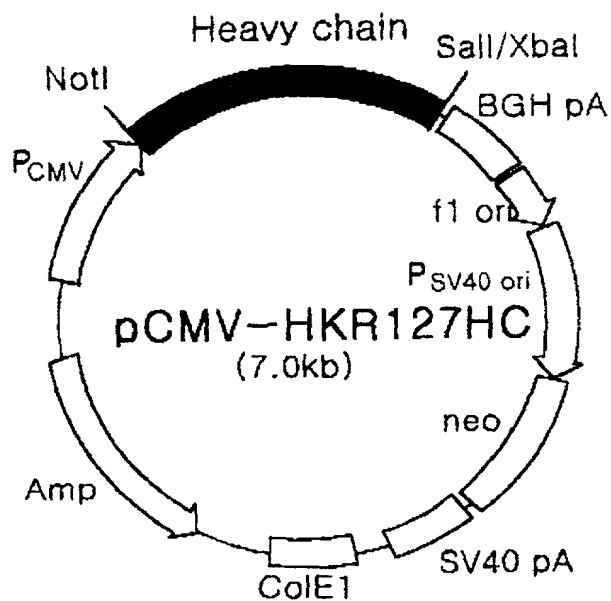


FIG. 5b

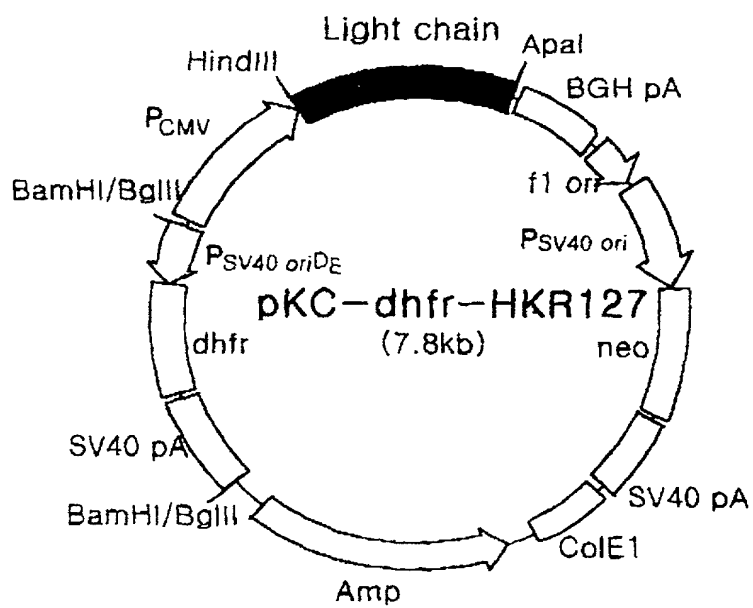
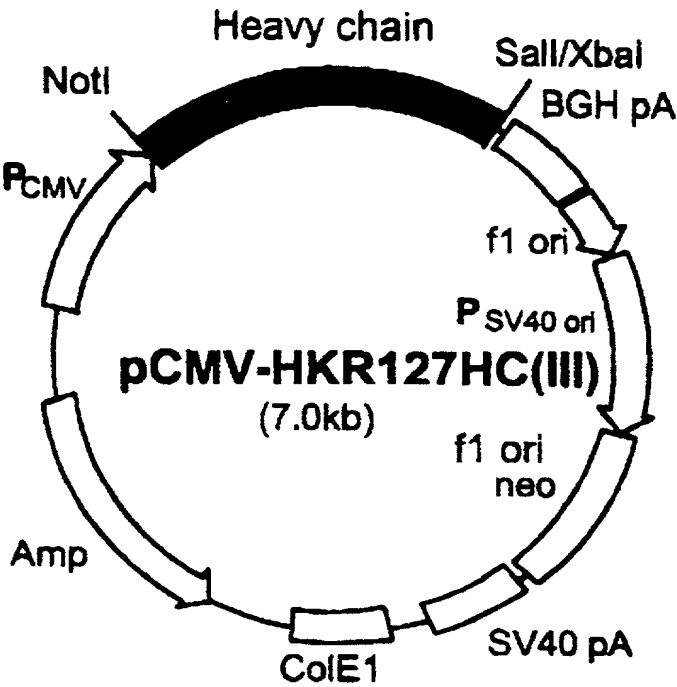
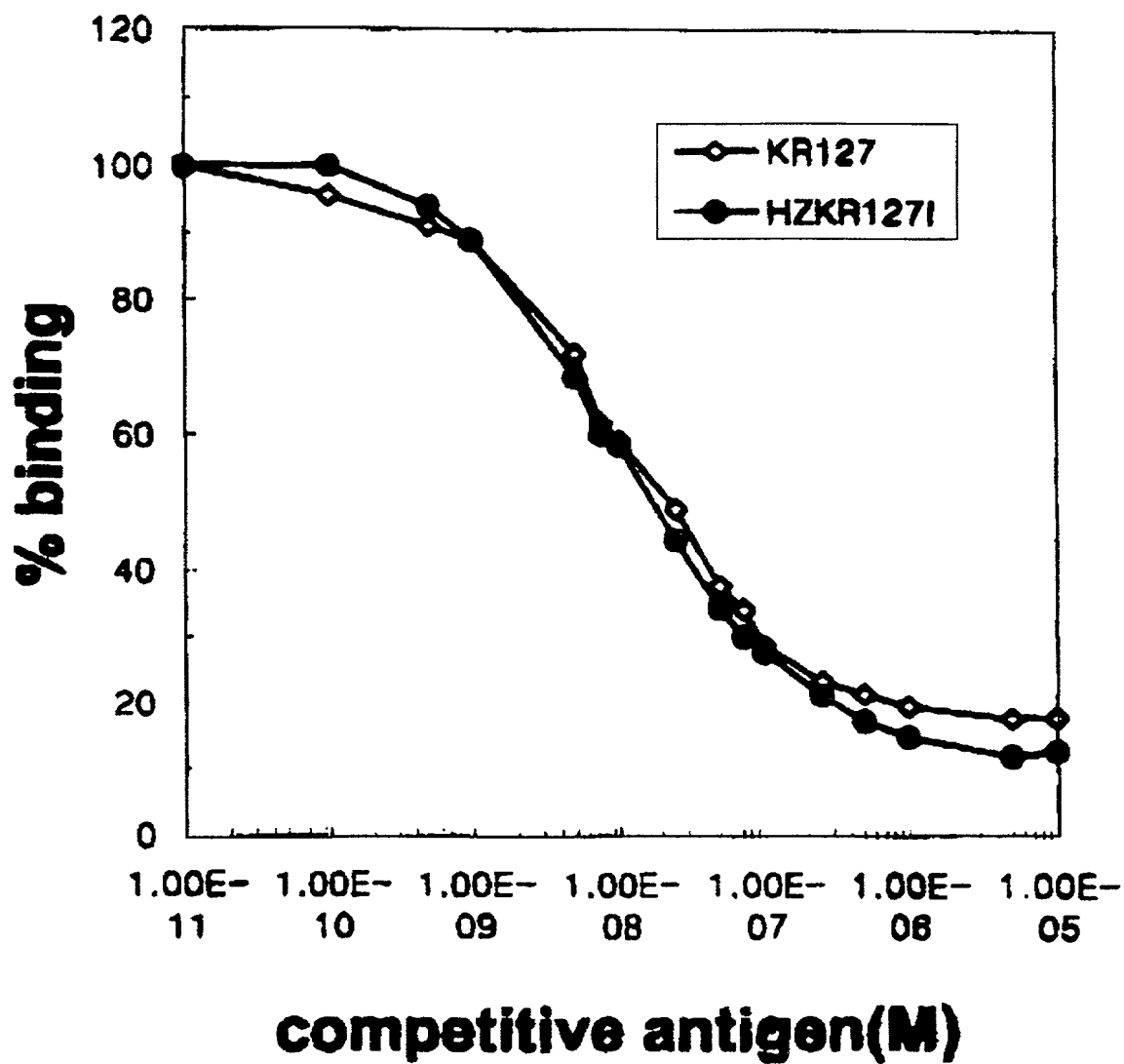


FIG. 5c



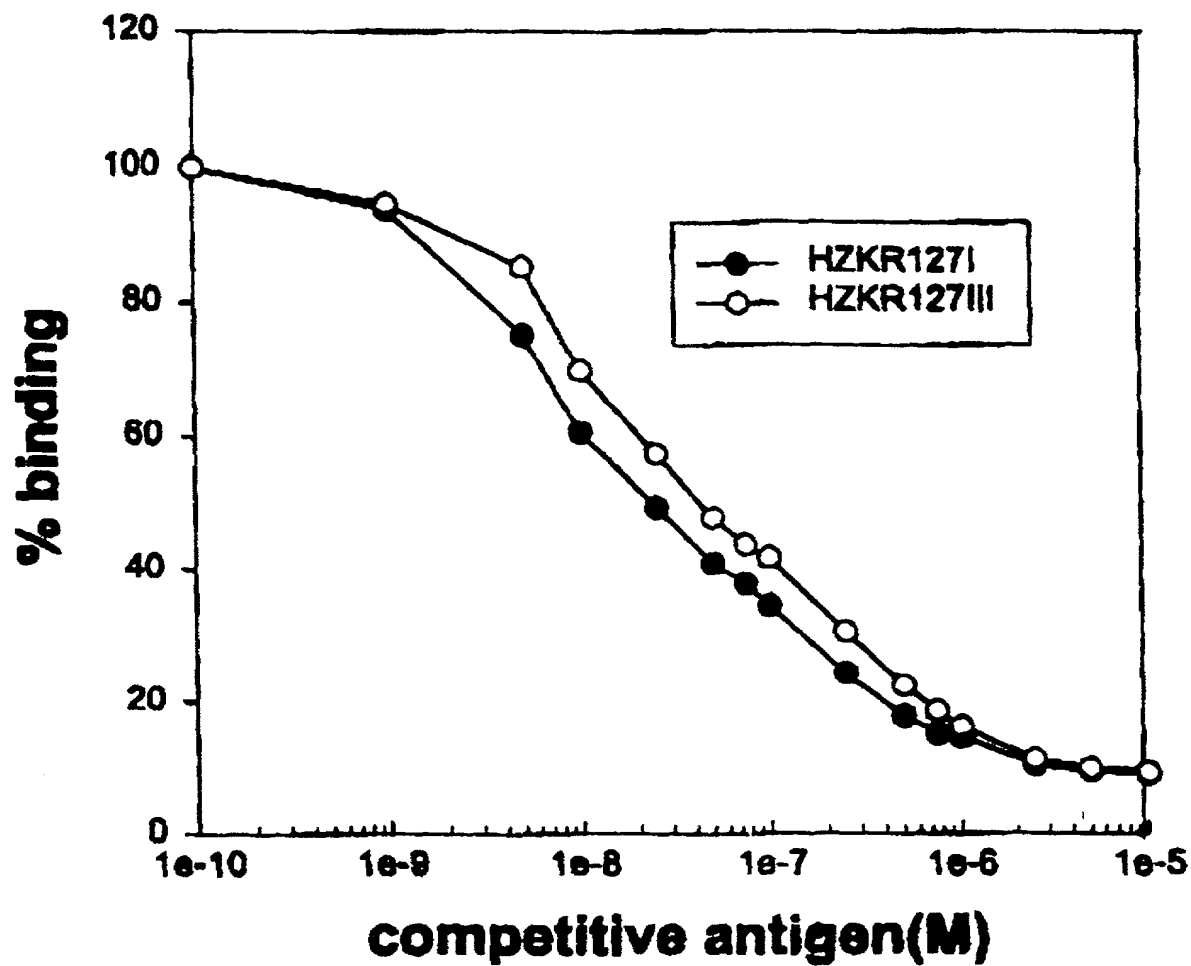
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FIG. 6a



09/856114

FIG. 6b



GATES & COOPER LLP

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV  
AND PREPARATION METHOD THEREOF

The specification of which was filed on November 19, 1999 as PCT International Application Number

PCT/KR99/00699

INTERNATIONAL FILING DATE

\_\_\_\_\_, which I have reviewed and for which I solicit a United States patent.  
INTERNATIONAL APPLICATION NUMBER

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT application having a filing date before that of the application on the basis of which priority is claimed:

- a. ☐ no such applications have been filed.  
b. ☐ such applications have been filed as follows:

| FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119           |                    |                                      |                                     |
|--|--------------------|--------------------------------------|-------------------------------------|
| COUNTRY  | APPLICATION NUMBER | DATE OF FILING<br>(day, month, year) | DATE OF ISSUE<br>(day, month, year) |
| KR   | 1998-49663         | November 19, 1998                    |                                     |
| OTHER FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S) |                    |                                      |                                     |
| COUNTRY  | APPLICATION NUMBER | DATE OF FILING<br>(day, month, year) | DATE OF ISSUE<br>(day, month, year) |
|  |                    |                                      |                                     |

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or 365(c) of any PCT international application(s) designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(G&C)

PCT-U.S. National Stage

| U.S. PARENT APPLICATION OR<br>PCT PARENT NUMBER | DATE OF FILING (day,<br>month, year) | STATUS (patented, pending,<br>abandoned) |
|---|--------------------------------------|--|
|   |                                      |  |

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

| U.S. PROVISIONAL APPLICATION NUMBER | DATE OF FILING (Day, Month, Year) |
|-------------------------------------|-----------------------------------|
|                                     |                                   |

I hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

|                  |                                |
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| George H. Gates  | Registration No. <u>33,500</u> |
| Victor G. Cooper | Registration No. <u>39,641</u> |
| Karen S. Canady  | Registration No. <u>39,927</u> |
| William J. Wood  | Registration No. <u>42,236</u> |
| Jason S. Feldmar | Registration No. <u>39,187</u> |
| Bradley K. Lortz | Registration No. <u>45,472</u> |

I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Gates & Cooper LLP to the contrary.

Please direct all correspondence in this case to the firm of Gates & Cooper LLP at the address indicated below:

~~GATES & COOPER LLP~~  
~~Howard Hughes Center~~  
 6701 Center Drive West, Suite 1050  
 Los Angeles, CA 90045

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

|   |  |   |   |
|---|--|---|---|
| (1) Full Name<br>Of Inventor                | Family Name<br><u>HONG</u>   | First Given Name<br><u>Hyo Jeong</u>          | Second Given Name                           |
| Residence<br>& Citizenship                  | City<br><u>Taejon-si</u>   | State or Foreign Country<br>Republic of Korea | Country of Citizenship<br>Republic of Korea |
| Post Office<br>Address                      | Post Office Address<br>#15-401 KIT Apt.,<br>237 Kajeong-dong,<br>Eusong-ku | City<br>Taejon-si                             | State & Zip Code/Country<br>305-350/KR      |
| Signature of Inventor(1): <u>M. J. Hong</u> |  |   | Date: <u>April 20, 2001</u>                 |

(G&C)

PCT to U.S. National Stage

|   |                         |   |   |   |
|---|-------------------------|---|---|---|
| (2)                                     | Full Name Of Inventor   | Family Name<br>RYU  | First Given Name<br>Chun Jeih                 | Second Given Name                           |
|   | Residence & Citizenship | City<br>Taejon-si   | State or Foreign Country<br>Republic of Korea | Country of Citizenship<br>Republic of Korea |
|   | Post Office Address     | Post Office Address<br>#136-1203 Hanbit Apt.,<br>99 Oeun-dong, Eusong-ku, | City<br>Taejon-si                             | State & Zip Code/Country<br>305-333/KR      |
| Signature of Inventor(2): Chun Jeih Ryu |                         |   |   | Date: April 20, 2001                        |
| (3)                                     | Full Name Of Inventor   | Family Name<br>HUR  | First Given Name<br>Hyangsuk                  | Second Given Name                           |
|   | Residence & Citizenship | City<br>Taejon-si   | State or Foreign Country<br>Republic of Korea | Country of Citizenship<br>Republic of Korea |
|   | Post Office Address     | Post Office Address<br>#106-1508 Expo Apt.,<br>Jeonmin-dong, Eusong-ku    | City<br>Taejon-si                             | State & Zip Code/Country<br>305-390/KR      |
| Signature of Inventor(3): Hyangsuk      |                         |   |   | Date: April 20, 2001                        |

1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

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(1) it establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) it refutes, or is inconsistent with, a position the applicant takes in:

(i) opposing an argument of unpatentability relied on by the Office, or

(ii) asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) each inventor named in the application:

(2) each attorney or agent who prepares or prosecutes the application; and

(3) every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

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SEQUENCE LISTING

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Chun Jeih Ryu  
Hangsook Hur

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